UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

March 8, 2022

MEMORANDUM

Subject: Efficacy Protocol Review for: **777PA9**
Product Name: Honey Cake Air Sanitization Efficacy Protocol Review
E-submission No.: 63770 / Submission No.: 1070144
Action Case Code: 00302989

From: Luisa C. Samalot-Freire
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Date signed: 3/8/2022

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Date signed: 3/8/2022

To: Marcel Howard / Stacey Grigsby PM34
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Applicant: Reckitt Benckiser, LLC
399 Interpace Parkway
Parsippany, NJ 07054

I. BACKGROUND

Reckitt Benckiser (RB) intends to determine efficacy of product following the EPA OCSPP Guideline 810.2500. Through the current submission, the registrant is submitting a new efficacy protocol for air sanitization entitled “RB Protocol to Assess Reduction in Bacterial Contamination in Indoor Air” and “RB Protocol to Assess Reduction in Viral Contamination in Indoor Air”.Protocols were developed by Reckitt Benckiser, LLC located at 399 Interpace Parkway, Parsippany,NJ 07054.

Documents considered in this review:

- Transmittal documentation to EPA dated 5/20/2021
- Cover letter from applicant to EPA dated 5/20/2021
- Proposed label language (excerpt) submitted with package (no date on document)
- Correspondence between EPA and Registrant dated 3/18/2021
- Whitepaper for submission to the U.S. EPA (Airborne Spread of SARS-CoV-2 and the Pandemic’s Potential Impacts on Human Health: Development of Technologies to Counter Virus Spread) dated 5/5/2021
- Two protocols submitted for review – one for bacteria and one for viruses (MRIDs 51534101 and 51534102)

II. BRIEF DESCRIPTION OF THE PROTOCOL - BACTERIA

Title: RB PROTOCOL TO ASSESS REDUCTION IN BACTERIAL CONTAMINATION IN INDOOR AIR

Purpose:
The purpose of this study is to evaluate the ability of a test substance to provide a temporary reduction in the number of bacteria in indoor air to support air sanitization labeling claims.

Method References:
ASTM International (2013). Annual Book of Standards. Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. Document #E2197. ASTM, Barr Harbor Drive, West Conshohocken, PA 1942.

Borges, J.T., L.Y.K. Nakada., M.G. Maniero, and J.R. Guimaraes. SARS-CoV-2: a systematic review of indoor air sampling for virus detection. Environ. Sci. Pollut. Res. 2021 Feb 25;1-14. doi: 10.1007/s11356-021-13001-w.

Centers for Disease Control and Prevention (2020). *Biosafety in Microbiological and Biomedical Laboratories*, 6th Edition,Publication No. 21-1112.
Dubuis et al. 2020. Ozone efficacy for the control of airborne viruses: Bacteriophage and norovirus models. https://doi.org/10.1371/journal.pone.0231164

Duchaine, C. 2016. Assessing microbial decontamination of indoor air with particular focus on human pathogenic viruses. http://dx.doi.org/10.1016/j.ajic.2016.06.009

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Fedorenko et al. 2020. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. https://doi.org/10.1038/s41598-020-79625-z

Ijaz, M.K., B. Zargar, K.E. Wright, J. Rubino, and S.A. Sattar. Generic aspects of the airborne spread of human pathogens indoor and emerging air decontamination technologies. Am. J. Infect. Control, 2016, 44(9 Suppl):S95-S101 http://www.ajicjournal.org/issue/S0196-6553(16)X0013-2

Kashkoli, F.M., Soltani, M, B. Zargar, J. Rubino, M.K. Ijaz, E. Taatizadeh, and S.A. Sattar. Analysis of an indoor air decontamination device inside an aerobiology chamber: a numerical-experimental study.Air Quality, Atmoshere & Health, 2019 / https://doi.org/10.1007/s11869-019-00782-w

Miles A.A., Misra S.S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg.* **38**: 732–749.

Organization for Economic Cooperation and Development (2013). Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces. OECD document No. ENV/JM/MONO(2013)11. OECD, Paris, France.
Prussin et al. 2018. Survival of the Enveloped Virus Phi6 in Droplets as a Function of Relative Humidity, Absolute Humidity, and Temperature. https://doi.org/10.1128/AEM.00551-18

Sattar, S.A., R.J. Kibbee, B. Zargar, K.E. Wright, J. Rubino, and M.K. Ijaz. Decontamination of indoor air to reduce the risk of airborne infections: Studies on survival and inactivation of airborne pathogens using an aerobiology chamber. Am. J. Infect. Control, 2016, 44(10): e177-e182 http://dx.DOI:10.1016/j.ajic.2016.03.067

Springthorpe, V.S. and Sattar, S.A. (2007). Application of a quantitative carrier test to evaluate microbicides against mycobacteria. J. AOAC International 90:817-824. Turgeon et al. 2014. Comparison of Five Bacteriophages as Models for Viral Aerosol Studies. http://doi:10.1128/AEM.00767-14

Zargar, F.M. Kaskooli, M. Soltani, K.E. Wright, M.K. Ijaz, and S.A. Sattar Mathematical modeling and simulation of bacterial distribution in an aerobiology chamber using computational fluid dynamics. Am. J. Infect. Control, 2016, 44(9 Suppl):S127-137 http://www.ajicjournal.org/issue/S0196-6553(16)X0013-2 Zargar, B., S.A. Sattar, J. Rubino, and

M.K. Ijaz. A quantitative method to assess the role of indoor air decontamination to simultaneously reduce contamination of environmental surfaces: testing with vegetative and spore-forming bacteria. Letters in Appl. Microbiol. 2019 / https://doi.org/10.1111/lam.13109

Test System (Microorganism):
Staphylococcus aureus (ATCC 6538)
Klebsiella pneumoniae (ATCC 4352)
Pseudomonas aeruginosa (ATCC 15442)
(Additional Bacteria may be listed)

Procedure:

Basic design of the aerobiology chamber:

- The aerosol chamber (Figure 1) is an enclosure with a volume of 900.0 ft^3 (25.00 M^3) located inside a clean room with negative pressure and controlled access. The chamber's walls are made out of wipe-able, solid coroplastic sheeting (https://www.homedepot.com/p/Coroplast-48-in-x-96-in-x-0-157-in-White-Corrugated-Plastic-Sheet-CP4896S/205351385) affixed to a framed structure to represent the walls to maintain an airtight seal. Sealable ports, window and door provide access to the inside of the chamber for maintenance and to place and remove any monitoring devices to be used. The walls should be grounded properly to dissipate any static electricity that may accumulate.
- In accordance with the current EPA guidelines (2012), the chamber does not permit any air exchanges; nor does it contain any furniture or fixtures in accordance with EPA 810.2500 study design description. Furniture and fixtures were not placed in the chamber inside of the BSL facility due to biosafety and decontamination concerns over the multiple test dates over a long period.
- The chamber's internal environment is monitored throughout an experiment with a wireless relative humidity (RH)/air temperature sensor/data logger system (e.g., CAS Data Loggers, 8437 Mayfield Rd., Unit 104 Chesterland, OH 44026); www.dataloggerinc.com/) and recorded on cloud for subsequent download and analysis.
- To assess the airborne survival of the test bacteria or to determine the activity of any air sanitization technology, the air in the chamber is sampled at the rate of 1 ft^3 (28.3 L/minute) using an externally-placed slit-to-agar air (STA) sampler with a built-in vacuum pump This programmable device can be set to operate for a minimum air sampling time of 30 seconds to as long as five hours depending on the STA model, and the actual length of sample collection time will be determined by the anticipated load of viable bacteria in the air of the chamber. The air exiting the sampler is discharged directly into a HEPA incorporated in the device or into the BSL-2 facility's HEPA-filtered exhaust system. For the baseline value, the concentration of the test bacteria in the nebulizer fluid should be adjusted to achieve a minimum of 4.2 $\log_{10}/m^3$ to a maximum of 5.0 $\log_{10}$ CFU/m^3 at the start of the treatment. An STA has been chosen due to its higher efficiency for sampling airborne microorganisms (Borges et al., 2021).
- Between experiments, the air inside the chamber is replaced with fresh air using a vacuum pump and the exiting air directly discharged into a BSC located in the clean room for a minimum of one hour.
- Start and stop times (clock times) will be recorded for the application of the treatment to the air. The official exposure period or contact time begins upon completion of the release of the test substance which should begin after the nebulizer has completed the 10-minute release of the test bacteria, five minutes for stabilization of the aerosols and the 2-minute pre-treatment air sample is taken.
- Any spray device can also be placed inside the chamber and activated from the outside or by accessing it with the gloves affixed to the chamber (Figure 1). The labeled use directions will be based upon the test substance application procedure used during testing.
- A magnehelic is affixed to the outside of the chamber to visually indicate on a continuing basis pressure differential between its internal and external atmospheres. Any pressure differential would be regarded as indicative of a breach in the integrity of the chamber resulting in the immediate termination of the test.

- The exposure period (contact time) may vary with the Test Substance. The same exposure period will be used to evaluate each lot of a Test Substance and controls. The air will be sampled for the same duration and at the same intervals for each lot of a Test Substance. The sampling will be continuous during the efficacy test and will continue for one hour after releasing the test substance. Sampling time for the control tests will be discrete and the period of sampling will be 2 minutes to get countable CFU on each plate, not fewer than five air samplings per microorganism will be collected.
- The air may be sampled for different durations and after different intervals for Test Substance and Controls to recover countable CFU on sampling plates and reduce the detection limit as much as possible. No fewer than three air samplings per lot per microorganism per chamber run will be collected for both Test substance and Controls. Each test lot will be evaluated in three runs of the chamber for each of the two bacterial species.

Experimental Design: A generic sequence of the main steps in the operation of the chamber is given in the Flowchart below.

Flowchart.

Day 1: Control Test

Switch on circulation fan;

Check environmental parameters and adjust as needed

Run an air sampler for 2 minutes for background contamination;

Nebulize bacteria for 10 minutes

Allow to stabilize for 5 minutes for uniform distribution of test microorganism in the chamber air

Collect another 2-minute Baseline air sample to confirm 4.2 -5.0 $\log_{10}$ CFU/m^3

Collect air samples for bacterial analyses at intervals listed in Table 3

Flush chamber with fresh air for at least one hour to decontaminate it;

Day 2: Efficacy Test

Switch on circulation fan;

Check environmental parameters and adjust as needed

Run an air sampler for 2 minutes for background contamination;

Nebulize bacteria for 10 minutes

Allow to stabilize for 5 minutes for uniform distribution of test microorganism in the chamber air

Collect another 2-minute Baseline air sample to confirm 4.2 -5.0 $\log_{10}$ CFU/m^3

Introduce test substance for 30 seconds inside the chamber

Collect air samples for bacterial analyses at intervals and duration listed in Table 2 to support claims

Flush chamber with fresh air for at least one hour to decontaminate it; Repeat for additional lots/controls

Study Acceptance Criteria:

- Test Substance Performance Criteria: After correction for bacterial settling and natural biological decay, the test substance must demonstrate ≥99.9% (3 $\log_{10}$) reduction in theviability of the bacterial species over the parallel untreated control.
- Baseline Acceptance Criteria: The control recovery must demonstrate a minimum of 4.2$\log_{10}$ to a maximum of 5.0 $\log_{10}$ CFU/m^3 at the start of the treatment for a valid test.
- The maximum allowable contact time to support an air sanitization claim should not be longer than 5 minutes for a >3 $\log_{10}$ reduction in the level of viability of all species of bacteria to be tested.

Control Acceptance Criteria:

- All sterility controls must be free of any visible growth.
- Viability Control must demonstrate growth in all media with <100 CFU/plate.
- Purity Control must demonstrate a pure culture.
- Neutralization Validation: The mean number of CFU on the plate unexposed to the test substance and those on the plate exposed to the test substance must be within 50%.
- Magnehelic readings must indicate no leaks in the chamber during an experiment.
- Temperature and RH readings must stay within range required for the test.

Retesting Guidance

For tests where the product passes and the mean Baseline value is above 5.0 $\log_{10}$ CFU/m^3, no retesting is necessary. For tests where the product fails and the mean *Baseline* is above 5.0 $\log_{10}$ CFU/m^3, retesting may be conducted. For tests where the product fails and the mean baseline is less than 4.2 $\log_{10}$ CFU/m_3, no retest is required.

III. BRIEF DESCRIPTION OF THE PROTOCOL - VIRUCIDAL

Title: RB PROTOCOL TO ASSESS REDUCTION IN VIRAL CONTAMINATION IN INDOOR AIR

Purpose:

The purpose of this study is to evaluate the ability of a test substance to provide a temporary reduction in the number of bacteriophages/viruses in an aerobiology chamber to support air treatment labeling claims.

Method References:

ASTM International (2013). Annual Book of Standards. Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. Document #E2197.

ASTM, Barr Harbor Drive, West Conshohocken, PA 1942. Borges, J.T., L.Y.K. Nakada., M.G. Maniero, and J.R. Guimaraes. SARS-CoV-2: a systematic review of indoor air sampling for virus detection. Environ. Sci. Pollut. Res. 2021 Feb 25;1-14. doi: 10.1007/s11356-021-13001-w. Centers for Disease Control and Prevention (2020). Biosafety in M icrobiological and Biomedical Laboratories, 6t h Edition, Publication No. 21-1112.

Dubuis et al. 2020. Ozone efficacy for the control of airborne viruses: Bacteriophage and norovirus models. https://doi.org/10.1371/journal.pone.0231164

Duchaine, C. 2016. Assessing microbial decontamination of indoor air with particular focus on human pathogenic viruses. http://dx.doi.org/10.1016/j.ajic.2016.06.009

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Fedorenko et al. 2020. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS CoV 2) in evaporated saliva microdroplets deposited on glass surfaces. https://doi.org/10.1038/s41598-020-79625-z

Ijaz, M.K., B. Zargar, K.E. Wright, J. Rubino, and S.A. Sattar. Generic aspects of the airborne spread of human pathogens indoor and emerging air decontamination technologies. Am. J. Infect. Control, 2016, 44(9 Suppl):S95-S101 http://www.ajicjournal.org/issue/S0196-6553(16)X0013-2

Kashkoli, F.M., Soltani, M, B. Zargar, J. Rubino, M.K. Ijaz, E. Taatizadeh, and S.A. Sattar. Analysis of an indoor air decontamination device inside an aerobiology chamber: a numerical-experimental study. Air Quality, Atmosphere & Health, 2019 / https://doi.org/10.1007/s11869-019-00782-w

Miles A.A., Misra S.S. (1938). The estimation of the bactericidal power of the blood. J. Hyg. 38: 732 749.

Organization for Economic Cooperation and Development (2013). Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces. OECD document No. ENV/JM/MONO(2013)11. OECD, Paris, France.

Prussin et al. 2018. Survival of the Enveloped Virus Phi6 in Droplets as a Function of Relative Humidity, Absolute Humidity, and Temperature. https://doi.org/10.1128/AEM.00551-18

Sattar, S.A., R.J. Kibbee, B. Zargar, K.E. Wright, J. Rubino, and M.K. Ijaz. Decontamination of indoor air to reduce the risk of airborne infections: Studies on survival and inactivation of airborne pathogens using an aerobiology chamber. Am. J. Infect. Control, 2016, 44(10): e177-e82 http://dx.DOI:10.1016/j.ajic.2016.03.067

Springthorpe, V.S. and Sattar, S.A. (2007). Application of a quantitative carrier test to evaluate microbicides against mycobacteria. J. AOAC International 90:817-824.

Turgeon et al. 2014. Comparison of Five Bacteriophages as Models for Viral Aerosol Studies. http://doi:10.1128/AEM.00767-14

Zargar, F.M. Kaskooli, M. Soltani, K.E. Wright, M.K. Ijaz, and S.A. Sattar Mathematical modeling and simulation ofbacterial distribution in an aerobiology chamber using computational fluid

dynamics. Am. J. Infect. Control, 2016, 44(9 Suppl):S127-137
http://www.ajicjournal.org/issue/S0196-6553(16)X0013-2

Zargar, B., S.A. Sattar, J. Rubino, and M.K. Ijaz. A quantitative method to assess the role of indoor air decontamination to simultaneously reduce contamination of environmental surfaces: Testing with vegetative and spore forming bacteria. Letters in Appl. Microbiol. 2019 / https://doi.org/10.1111/lam.13109

Test Microorganism*:

Virus (ATCC #)	Host cell & Incubation	Justification
MS-2 (15597-B1)	*Escherichia coli* (15597); 36±1°C	Small-sized (~30 nm), non-enveloped with RNA genome; often used as a surrogate for non-enveloped human pathogenic viruses (e.g., noro- and rhino-viruses)
Phi6 (4352-B1)	*Pseudomonas syringae* (31952); 30±1°C	Medium-sized (~100 nm), enveloped with RNA genome; often used as a surrogate for enveloped human pathogenic viruses (e.g., corona- and influenza viruses)

*As presented on Table 1 of the proposed protocol (page 6)

Procedure:

Basic design of the aerobiology chamber:

- Appendix 1 summarizes the details on the specialized pieces of equipment used in the protocol. The equipment and materials listed are examples only and may be substituted with equivalent items from other sources.
- The aerosol chamber (Figure 2) is an enclosure with a volume of 900.0 ft^3 (25.00 m^3) located inside a clean room with negative pressure and controlled access. The chamber's walls are made out of wipe-able, solid coroplastic sheeting (https://www.homedepot.com/p/Coroplast-48-in-x-96-in-x-0-157-in-White-Corrugated-Plastic-Sheet-CP4896S/205351385) affixed to a framed structure to represent the walls to maintain an airtight seal. Sealable ports, window and door provide access to the inside of the chamber for maintenance and to place and remove any monitoring devices to be used. The walls should be grounded properly to dissipate any static electricity that may accumulate (Sattar et al 2016).
- While the chamber can be used with all major classes of microorganisms at biosafety levels (BSL) 1 and 2, the CDC guidelines (CDC 2020) recommend that the extra safety precautions and operational requirements be in place for work with experimental aerosols of all such microorganisms. Therefore, the aerobiology chamber is house inside a room with negative pressure and controlled access. This elevates the biosafety containment level of the CREM Co facility to 'BSL-2+'.
- In accordance with the current EPA guidelines (2012), the chamber does not permit any air exchanges, nor does it contain any furniture or fixtures in accordance with EPA

810.2500 study design description. Furniture and fixtures were not placed in the chamber inside of the BSL facility due to biosafety and decontamination concerns over the multiple test dates over a long period.

- The chamber's internal environment is monitored throughout an experiment with a wireless relative humidity (RH)/air temperature sensor/data logger system (e.g., CAS Data Loggers, 8437 Mayfield Rd., Unit 104 Chesterland, OH 44026); www.dataloggerinc.com/) and recorded on cloud for subsequent download and analysis.
- To assess the airborne survival of the test bacteriophages / viruses or to determine the activity of any air treatment technology, the air in the chamber is sampled at the rate of 28.3 L/minute using an externally placed slit-to-agar air (STA) sampler with a built-in vacuum pump. This programmable device can be set to operate for a minimum air sampling time of 30 seconds to as long as five hours depending to the STA model, and the actual length of sample collection time will be determined by the anticipated load of viable bacteriophage / viruses in the air of the chamber. The air exiting the sampler is discharged directly into a HEPA incorporated in the device or into the BSL-2 facility's HEPA-filtered exhaust system. For the baseline value, the concentration of the test bacteriophage / viruses in the nebulizer fluid should be adjusted to achieve a minimum of 4.2 $\log_{10}$ to a maximum of 5.0 $\log_{10}$ PFU per m^3 at the start of the treatment. Here it should be noted that a recent review indicated that semi-solid impactors are more effective than liquid impingers for air sampling for virus detection (Borges et al., 2021). That is why CREM Co Labs prefers using STA for air sampling.
- Between experiments, the air inside the chamber is replaced with fresh air using a vacuum pump and the exiting air directly discharged into a BSC located in the clean room for a minimum of one hour.
- The Start and Stop times (clock times) will be recorded for the application of the treatment to the air. The official exposure period or contact time begins upon completion of the release of the test substance which should begin after the nebulizer has completed the 10-minute release of the test bacteriophage / virus, five minutes for stabilization of the aerosolized microorganism and the 2-minute pre-treatment air sample is taken.
- Any spray-formulation can also be placed inside the chamber and activated from the outside or by accessing it with the gloves affixed to the chamber (Figure 1). The labeled use directions will be based upon the test substance application procedure used during testing.
- A magnehelic is affixed to the outside of the chamber to visually indicate on a continuing basis pressure differential between its internal and external atmospheres. Any pressure differential would be regarded as indicative of a breach in the integrity of the chamber resulting in the immediate termination of the test.
- The Contact Time of a Test Substance in aerobiology is defined as the time required for the Test Substance to demonstrate the desired level of reduction {≥3.0 $\log_{10}$ reduction of the test microbe(s). Appropriate sampling duration and interval should be determined during R&D tests before GLP studies. Getting countable colonies on the sampling plates and having the minimum limit of detection are two important factors which determine the appropriate sampling duration and intervals between sample collections. The air will be sampled for the same duration and at the same intervals for each lot of a Test Substance. The sampling will be continuous during the efficacy test, to improve the limit of detection and will continue for one hour after releasing the test substance. Sampling time for the control tests will be

discrete and the period of sampling will be 2 minutes to get countable PFU on each air sample plate, not fewer than five air samplings per microorganism will be collected in any given test.

- The air may be sampled for different durations and after different intervals for Test Substance tests and Controls to recover countable PFU on sampling plates and improve the limit of detection as much as possible. No fewer than five air samplings per lot per microorganism per chamber run will be collected for both Test Substance and Controls. Each test lot will be evaluated in three runs of the chamber for each one of the two bacteriophages.

  Experimental Design: A generic sequence of the main steps in the operation of the chamber is given in the Flowchart below.

Flowchart

Day 1: Control Test

Switch on circulation fan;

Check environmental parameters and adjust as needed

↓

Run an air sampler for 2 minutes for background contamination;

↓

Nebulize bacteriophage / virus for 10 minutes

↓

Allow to stabilize for 5 minutes for uniform distribution of test microorganism in the chamber air

↓

Collect another 2-minute Baseline air sample to confirm 4.2 -5.0 $\log_{10}$ PFU/m^3

↓

Collect air samples for bacterial analyses at intervals listed in Table 3

↓

Flush chamber with fresh air for at least one hour to decontaminate it;

Day 2: Efficacy Test

Switch on circulation fan;

Check environmental parameters and adjust as needed

Run an air sampler for 2 minutes for background contamination;

Nebulize bacteriophage / virus for 10 minutes

Allow to stabilize for 5 minutes for uniform distribution of test microorganism in the chamber air

Collect another 2-minute Baseline air sample to confirm 4.2 -5.0 log_{10} PFU/m^3

Introduce test substance for 30 seconds inside the chamber

Collect air samples for bacterial analyses at intervals and duration listed in Table 2 to support claims

Flush chamber with fresh air for at least one hour to decontaminate it; Repeat for additional lots/controls

Study Acceptance Criteria:

- Test Substance Performance Criteria: After correction for aerosol settling and natural biological decay, the test substance must demonstrate ≥99.9% (3.0 log_{10}) reduction in the viability of each bacteriophage / virus over the parallel untreated control.

- If cytotoxicity is present, the virus/bacteriophage titer should be increased if necessary to demonstrate a ≥3 log_{10} reduction in PFU/m_3 beyond the cytotoxic level.
- Baseline Acceptance Criteria: The control recovery must demonstrate a minimum of 4.2 log_{10} to a maximum of 5.0 log_{10} PFU/m_3 at the start of the treatment for a valid test.
- The maximum allowable contact time to support an air treatment claim should not be longer than 5 minutes for a ≥3 log_{10} in the level of viability of all species of bacteriophage/viruses to be tested.

Control Acceptance Criteria:

- All sterility controls must be free of any visible growth.
- Viability Control must demonstrate growth in all media with <100 PFU/plate.
- Purity Control must demonstrate a pure culture.
- Neutralization Validation: The mean number of PFU on the plate unexposed to the test substance and those on the plate exposed to the test substance must be within 50%.
- Magnehelic readings must indicate no leaks in the chamber during an experiment.
- Air temperature and RH readings must stay within range required for the test.

Retesting Guidance:

For tests where the product passes and the mean Baseline value is above 5.0 log_{10} PFU/m^3, no retesting is necessary. For tests where the product fails and the mean baseline is above 5.0 log_{10} PFU/m^3, retesting may be conducted. For tests were the product fails and the mean baseline is less than 4.2 log_{10} PFU/m^3, no retest is required.

IV. CONCLUSION

1. The submitted protocol (MRID 51534101 and MRID 51534102) **is adequate** for assessing the reduction of air bacterial and viral concentration. However, this does not guarantee full acceptance of the efficacy data generated using this protocol.
2. As stated on the Agency Air Sanitizing Guidance 810.2500, contact times of 5 minutes or less are to be considered for Air Sanitization for bacteria. Contact times above 10 minutes, such as the contact time proposed for viruses can carry an air treatment claim (per the correspondence with EPA Antimicrobial Division Director and Efficacy Branch Chief, dated 3/16/2021).
3. This protocol is a spray (trigger) formulation, in order to provide the user consistency Use Directions should indicate the number of pumps or the duration of pumps or sprays per a determined amount of time to ensure adequate room coverage and thus appropriate log reduction.
4. Since the user must be in the room during application, it is advised to add necessary information regarding PPE or respiratory protection.
5. For treatment of rooms equipped with HVAC system, returns and registers must be closed or sealed (and shut air system down if possible).

6. It is advised that Directions of Use be specific on how to set up the room prior to product application. Expertise may be required to seal a room completely, depending on the complexity of the room being treated.
    a) It should be taken into consideration that closing all doors, windows and air vents may not be feasible for all ventilation systems and that even with these precautions air circulation may continue during application due to air registers within the HVAC systems. Certain HVAC systems may not be fully turned off, in this case a pre-cautionary note regarding air circulation should be added to the label.
    b) It is suggested to add pre-cautionary language regarding adjacent rooms as this rooms may have direct or indirect exposure to aerosolized product particles.
7. On the label it is advised to specify the amount of time that must pass before re-entering the room after product application. The current statement: “Resume to normal ventilation after spray has settled” is too broad, thus increasing unnecessary room occupant exposure to product aerosolized particles. In addition, the user may not be able to identify when the spray has settled, thus creating potential unnecessary exposure to the pesticide.
    a) In addition, specify that room must be un-occupied for the duration of product application and for x amount of time after product has settled.
8. On the label it is advised to provide clarification on the room size. Currently the room size is defined as a 10 ft x 10 ft x 8 ft, we recommend adding instructions for rooms of other sizes and how to adjust product application accordingly.
9. On the label under Use Directions, the text “Repeat as necessary” should be qualified and clarified. They user may not be able to know that the air has been treated or for how many times the product should be applied in order to achieve proper air treatment.
10. On the label, under Use Sites – the word “Surfaces” may be misleading as this is not a surface treatment product, suggest a footnote associated with this statement to specify that the product is not meant to treat surfaces, but rather be used as an air sanitizer or air treatment in relation to the suggested contact time.
11. It is a reminder that product lots must be tested at the LCL. The lowest effective air treatment concentration must be used.
12. The method lacks time matched controls. In lieu of such, “baseline” samples are collected at 5 minutes post-neutralization. Further the bactericidal stability in air control omits treatment with the test substances to measure survival/settling rate.
13. Relative humidity (RH) variability between test and stability controls should be considered to ensure that control conditions are appropriate and assess efficacy independent of RH.